

Effect of the Hot Water Extract of *Artocarpus camansi* Leaves on 2,4,6-Trinitrochlorobenzene (TNCB)-Induced Contact Hypersensitivity in Mice

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Medicinal plants with reported anti-inflammatory activity could have the potential use as anti-allergens and inhibitors of allergic contact dermatitis reactions produced by allergens and chemicals. Some species from the genus *Artocarpus* were reported to have anti-inflammatory activity. In the Philippines one local source is *Artocarpus camansi* BLANCO (Moraceae), which is utilized as an ingredient of their cuisine, and decoction of leaves is used for diabetes and baths of people with rheumatism. The objective of this study was to evaluate the effect of the hot water extract of *A. camansi* leaves on contact hypersensitivity (CHS) in mice. Contact hypersensitivity was induced using 2,4,6-trinitrochlorobenzene (TNCB). The results showed that the *A. camansi* hot water extract exhibited significant activity against the swelling produced during 24 h and 48 h post-challenge. The same responses were observed from the mice that received the kamansi ethanol-precipitate (KEP) and kamansi ethanol precipitate water-soluble (KEPWS) fractions. Since the high molecular mass fraction showed the significant activity, we therefore speculate that the compound responsible might be a polysaccharide and/or glycoprotein. In conclusion, our results suggest that the hot water extract of *A. camansi* leaves might be an effective natural product to treat allergic contact dermatitis. However, further investigations are required to understand the mechanisms involved.

Key words *Artocarpus camansi*; Moraceae; contact hypersensitivity; regulatory T cell; ethanol precipitation

Allergic contact dermatitis (ACD) is a common occupational skin disease and environmental health issue in industrialized countries with a great socio-economic impact.^{1,2)} It is a form of delayed-type hypersensitivity characterized by redness, papules and vesicles, followed by scaling and dry skin. ACD is an inflammatory reaction mediated by T cell occurring at the site of challenge with a contact allergen, such as hapten, in sensitized individuals. The immunological mechanisms of ACD in humans can be studied chiefly from animal models referred to as contact hypersensitivity (CHS). It provides the option of studying many aspects of the immune system and basic immunological mechanisms *in vivo*, which is relevant not only for ACD but for a whole range of inflammatory diseases.³⁾

Glucocorticoids are the more common treatment for ACD but they have high level of undesirable effects. Most patients especially in developing countries, like the Philippines, still resort to Complementary and Alternative Medicine (CAM). This includes the use of herbal remedies, proving that plants are still one of the most important tools in drug research. Medicinal plants with reported anti-inflammatory activity could have the potential use as anti-allergens and inhibitors of ACD reactions produced by allergens and chemicals.⁴⁾ Some species from the genus *Artocarpus* were reported to have anti-inflammatory activity.⁵⁾ They are used as traditional folk medicine in South-East Asia for the treatment of inflammation, malarial fever, and to treat the ulcers, abscess and diarrhea.^{6,7)}

In the Philippines one local source is *Artocarpus camansi* BLANCO (Moraceae), common name is “breadnut” in English and “kamansi” in Filipino, which is utilized as an ingredient of their cuisine and decoction of leaves is used for diabetes and baths of people with rheumatism, but the pharmacological property of *Artocarpus camansi* has not been reported. There-

fore, this study was carried out to evaluate the effect of the hot water extract of *A. camansi* leaves on contact hypersensitivity in mice.

MATERIALS AND METHODS

Plant Material Matured leaves of *A. camansi* were gathered and selected from Apalit, Pampanga Philippines in January 2012. Samples were submitted to the University of Santo Tomas Herbarium Research Section of the Research Center for Natural and Applied Sciences at Tomas Aquinas Research Complex (España, Manila, Philippines) and were identified and authenticated by Rosie S. Madulid with the account number USTH 5759.

Animals Six-week old female BALB/c mice, purchased from Japan SLC (Hamamatsu, Japan), were used in the study. The mice were housed in standard plastic cages in a temperature- and humid-controlled environment, with food and water available *ad libitum*. A period of at least 7 d of acclimatization was allowed prior to experimentation. All experiments were carried out following the guideline for the care and use of experimental animals made by the Animal Care Committee of the Faculty of Pharmacy, Meijo University.

Preparation of the Hot Water Extract and Its Fractions The leaves were cleansed from dust particles and air-dried before cutting and milling. Fifty grams milled leaves were placed in a tea maker with 1L-distilled water and was heated for 60 min. Then the extract was filtered, and the residue was re-extracted following the same procedure. For the last extraction, 500 mL of distilled water was added and the same procedure was done. The extracts were combined and then lyophilized to powder. The yield of the extract was 20.6 percent (w/w).

In order to fractionate the powdered hot water extract into high molecular mass fraction and low molecular mass frac-

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tion the ethanol precipitation was carried out.⁸⁾ The powdered extract was dissolved in water and added with 3 volumes of EtOH to precipitate the solution giving Kamansi EtOH-soluble (KES) and Kamansi EtOH-precipitate (KEP). The KEP was suspended in the appropriate volume of water and lyophilized to powder (30.8%), while the KES was concentrated *via vacuo* and then lyophilized to powder (69.2%).

The powdered KEP was dissolved in water and then was centrifuged at $9000\times g$ for 15 min. Both the water-soluble fraction (KEPWS) and insoluble fraction (KEPWIS) were lyophilized to powder (31.0% and 65.2% yield, respectively). The freeze-dried extract and the fractions were reconstituted with distilled water just before administration of animals.

Contact Hypersensitivity The mice were sensitized by painting their shaven abdominal skin with $100\mu\text{L}$ of 5% 2, 4, 6-trinitrochlorobenzene (TNCB) in acetone. The CHS response was elicited 7d later by painting both surfaces of the right ear of each mouse with $20\mu\text{L}$ 1% TNCB in olive oil. The ear thickness was measured before and after (24 and 48h) the challenge using a dial thickness gauges (G-1A, Ozaki Mfg., Co., Ltd., Tokyo Japan). Oral administration of the crude hot water extract (HWE) at various doses (100, 500 and 1000mg/kg body weight (BW)) was started right after the mice were sensitized once daily for 7d. Prednisolone (20mg/kg BW) was used as the positive control drug. Mice that received the same dose of TNCB for skin sensitization as well as elicitation on ear skin and received distilled water served as CHS control group, whereas the mice that were painted with acetone and challenged with olive oil served as normal control group. The same protocol was used for the animals that were given KES, KEP, KEPWS and KEPWIS with an equivalent dose of the hot water extract (500mg/kg BW).

Adoptive Transfer The donor mice were sensitized with 5% TNCB and were given with KEPWS or distilled water. Seven days after sensitization, the mice were sacrificed and lymphocyte suspensions were prepared from the draining lymph nodes (inguinal and axillary) for use in the subsequent experiments. In the first set of experiments, to determine the effect on effector T cell function, the cells (1×10^7) in $100\mu\text{L}$ of Hanks' balanced salt solution (HBSS) obtained from the donor mice were injected intravenously into each naïve recipient mice. The mice were challenged 24h later with 1% TNCB on the ear skin. In the second set of experiments, to determine the effect on regulatory T cell function, the cells (1×10^7) were injected intravenously into each naïve recipient mice and they were sensitized 24h after injection of the cells and challenged 7d after. For each experiment, ear thickness was measured before and after (24 and 48h) the challenge. In both experiments, the vehicle (HBSS)-injected recipient mice served as vehicle (V)-negative control group.

Statistical Analysis Results are given as mean $\pm$ S.E.M. Statistical analysis was conducted by ANOVA and Bonferroni's multiple type *t*-tests.

RESULTS AND DISCUSSION

Consumption of herbal medicines is widespread and increasing significantly in both traditional and modern medicine. According to the estimation of the World Health Organization (WHO), more than 80% of the world's population in developing countries depends primarily on herbal medicine

for basic healthcare needs.⁹⁾

The genus *Artocarpus* is comprise of about 50 species of evergreen and deciduous trees and it is an important source of edible fruit, timber and folkloric medicinal use. *Artocarpus camansi* is a medium-sized tree found in the mulberry family, Moraceae and is native to Papua New Guinea and possibly the Moluccas (Indonesia) and the Philippines. It is a relative of the breadfruit, *Artocarpus altilis*, and is commonly used as a staple crop. In folkloric medicine, the decoction of *A. camansi* bark is used as vulnerary and for dysentery. Crushed leaves are used for thrush and juice from stems of leaves for ear infections. In the West Indies, decoction of yellowing leaf is used for high blood pressure and asthma and the tea is also used for diabetes.^{10,11)} Here, we examined the immunopharmacological action of the hot water extract of leaves of *A. camansi* on TNCB-induced contact hypersensitivity in mice.

When the hot water extract of *A. camansi* leaves was given for 7d by oral administration from the sensitization, prednisolone (20mg/kg BW) and various doses of the extract (100, 500 and 1000mg/kg BW) inhibited the ear swelling at 24h after the challenge compared with the control group. Furthermore, prednisolone and 1000mg/kg extract still inhibited the ear swelling at 48h after the challenge (Fig. 1). Since there were no significant differences between the three doses of the extract given, 500mg/kg BW and the different fractions with an equivalent dose of the hot water extract were used in the subsequent experiments. In our series of studies that aims to provide scientific bases to the pharmacological properties of Philippine medicinal plants, we found out that the hot water extract of *A. camansi* leaves reduced the TNCB-induced contact hypersensitivity in mice.

In order to determine the active compound responsible for the anti-allergic activity of the hot water extract, we fractionated the extract into two (KES and KEP) using ethanol precipitation. As shown in Fig. 2, the Kamansi ethanol precipitate (KEP) fraction and the crude extract showed significant reduction of ear swelling at 24h and 48h post challenge, whereas KES fraction did not. Since the KEP fraction contains water-soluble and insoluble materials, we further fractionated it into a water-soluble fraction labeled as KEPWS and water insoluble fraction labeled as KEPWIS. The KEPWS has significant suppressive effect in mice ear swelling at 24h and 48h after the challenge whereas KEPWIS did not exhibit anti-allergic effect (Fig. 3). These results suggest that the active ingredients may be water-soluble macromolecular compounds such as polysaccharide and/or glycoprotein.

To elucidate the anti-allergic mechanism of the hot water extract of *A. camansi* leaves on TNCB-induced contact hypersensitivity, the adoptive transfer experiments were carried out. We administered the active fraction (KEPWS) to the donor mice in these experiments. The draining lymph node cells from donor mice that had been sensitized 7d earlier by topical treatment of 5% TNCB in acetone on the abdominal skin, with or without administration of KEPWS, were injected i.v. into naïve recipient mice. In the first set of experiments, the mice were challenged 24h later by application of 1% TNCB onto the ear skin and ear swelling was measured 24h and 48h later. The ear swelling of the naïve mice that received cells from the mice that were treated with KEPWS did not show any significant difference compared to the control group (Fig. 4A). On the other hand, in the second set of experiments,

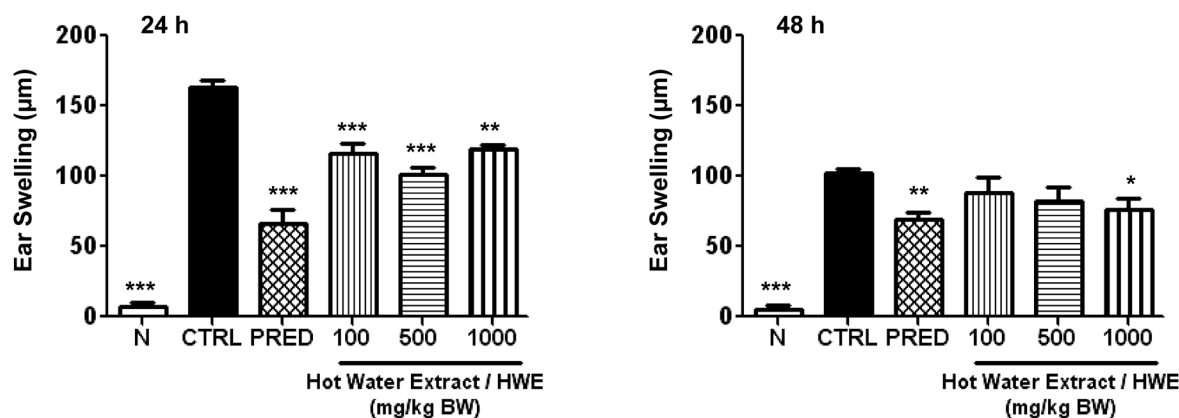


Fig. 1. Effect of the Hot Water Extract of *Artocarpus camansi* BLANCO Leaves on TNCB-Induced Contact Hypersensitivity in Mice

Distilled water was given to the normal (N) and control (CTRL) group. Each column represents mean $\pm$ S.E.M. of 5 mice. *** p <0.001, ** p <0.01, * p <0.05 versus the control group by ANOVA and Bonferroni's type multiple t -test.

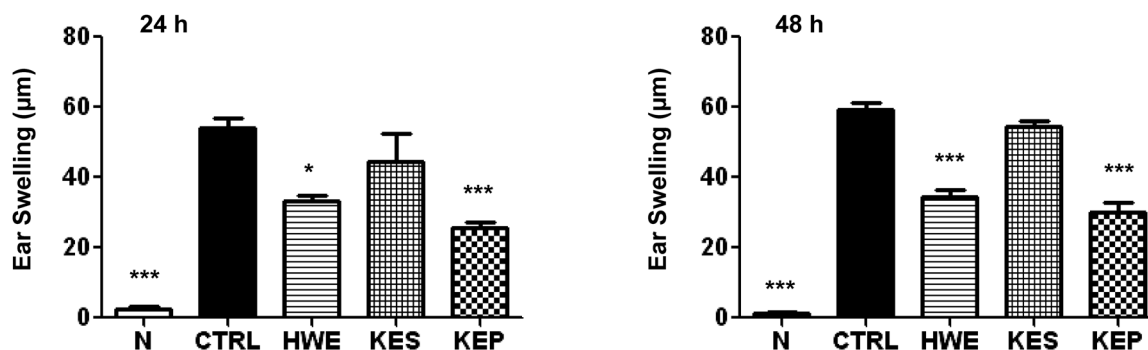


Fig. 2. Effect of the Hot Water Extract of *Artocarpus camansi* BLANCO Leaves and Its Fractions on TNCB-Induced Contact Hypersensitivity in Mice

Distilled water was given to the normal (N) and control (CTRL) group. Hot water extract of *Artocarpus camansi* BLANCO leaves (HWE) was administered orally once a day for 7 d at a dose of 500 mg/kg. The fractions, KES and KEP, were also administered orally once a day for 7 d at a dose of 346 mg/kg and 154 mg/kg (equivalent doses of 500 mg/kg HWE), respectively. Each column represents mean $\pm$ S.E.M. of 5 mice. *** p <0.001, * p <0.05 versus the control group by ANOVA and Bonferroni's type multiple t -test.

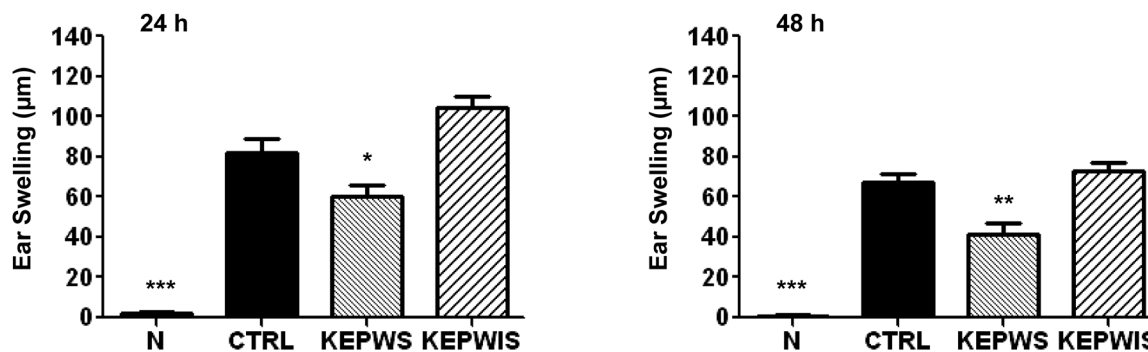


Fig. 3. Effect of KEP Water-Soluble (KEPWS) and KEP Water-Insoluble (KEPWIS) Fractions of the Hot Water Extract of *Artocarpus camansi* BLANCO Leaves on TNCB-Induced Contact Hypersensitivity in Mice

Distilled water was given to the normal (N) and control (CTRL) group. Each fraction was administered orally once a day for 7 d at a dose of 50 mg/kg KEPWS and 100 mg/kg KEPWIS (equivalent doses of 500 mg/kg HWE), respectively. Each column represents mean $\pm$ S.E.M. of 5 mice. *** p <0.001, ** p <0.01, * p <0.05 versus the CTRL group by ANOVA and Bonferroni's type multiple t -test.

the recipient mice that were challenged 7 d after the draining lymph node cells from the treated donor mice was injected exhibited lower CHS response than the control mice at 24 and 48 h post-challenge (Fig. 4B). These results suggest that the fraction did not affect the effector T cell function but it suppressed the TNCB-induced contact hypersensitivity through

the activation of regulatory T cell function. Regulatory T cells play crucial roles in the maintenance of immunological self-tolerance and immune homeostasis. Most especially the naturally occurring CD4⁺CD25⁺FoxP3⁺ T cells that are produced by the thymus, and could also be induced at the periphery, are capable of recognizing both self and non-self

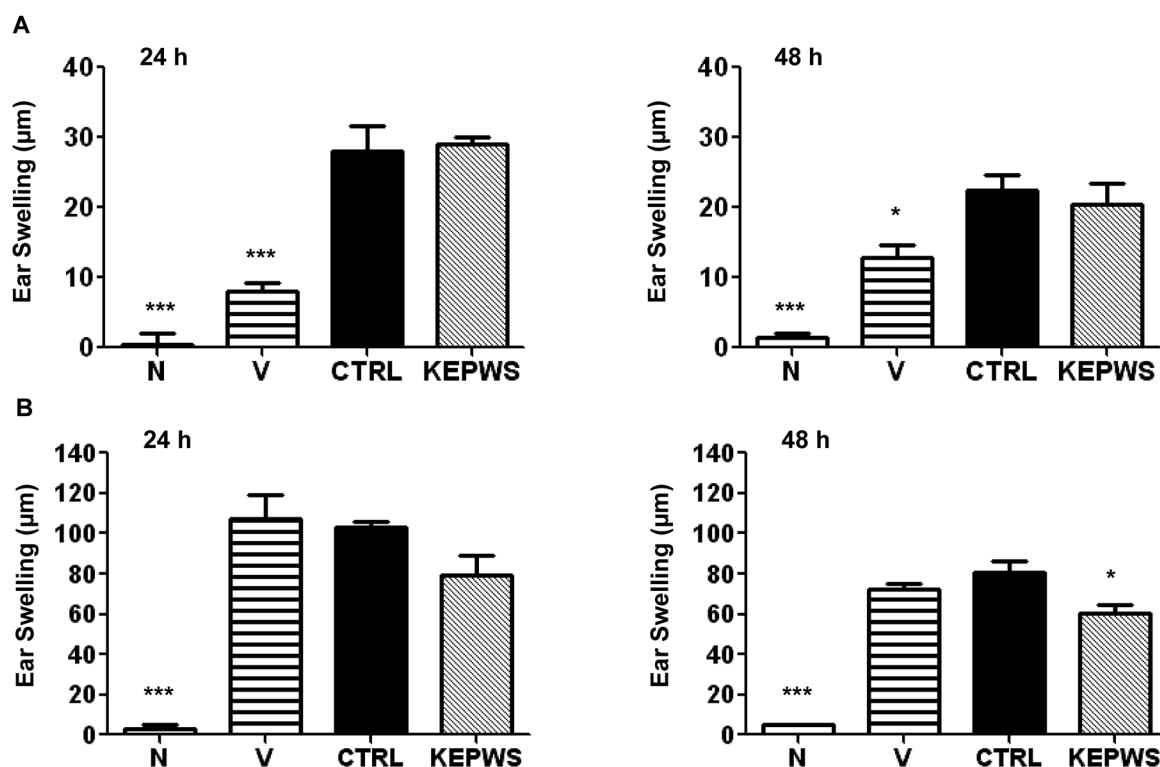


Fig. 4. Effect of KEPWS Fraction on Effector T Cell Activity (A) and Regulatory T Cell Activity (B) on TNCB-Induced Contact Hypersensitivity in Mice

In donor mice, KEPWS fraction was administered orally once daily at a dose of 50 mg/kg to the treated group and distilled water was given to the control (CTRL) group right after they were sensitized with 5% TNCB. Seven days later lymphocytes were obtained from the draining lymph nodes and were injected into naïve recipient mice. The lymphocytes obtained from the control donor mice were injected intravenously into the CTRL group and lymphocytes obtained from the KEPWS-treated donor mice were injected intravenously into the KEPWS group. HBSS alone was injected intravenously in the vehicle (V) group. All mice except the normal (N) group were then sensitized with 5% TNCB and ear challenged with 1% TNCB on day 7 (B). Ear swelling was determined at 24 and 48 h after challenge. Each column represents mean $\pm$ S.E.M. of 5 mice. *** $p < 0.001$, * $p < 0.05$ versus the CTRL group by ANOVA and Bonferroni's type multiple t -test.

antigens.^{12,13}) It has been reported that UV irradiation and some kinds of probiotics can also induce regulatory T cells and suppress hapten-induced allergic contact dermatitis. Moreover, the injection of lymph node cells containing T cells obtained from UV-irradiated and hapten-treated mice 4 d after treatment into naïve syngeneic mice resulted in transfer of suppression observed during ear challenge performed 5 d after injection.^{14,15}) Through the adoptive transfer experiments it is very interesting to find that the plant extract might possess the same anti-allergic action. Additional studies on characterization of regulatory T cells induced/activated by the hot water extract of *A. camansi* leaves and how these regulatory T cells suppress contact hypersensitivity are still needed.

In conclusion, the hot water extract of *A. camansi* leaves might be an effective natural product to treat allergic contact dermatitis. The lack of progressive research to prove the efficacy of a plant could be one of the major reasons why herbal medicine is still limited for clinical use in the Philippines. Our results suggest that *A. camansi* can be a valuable treatment for allergic contact dermatitis. However, further investigations are required to understand the mechanisms involved.

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